



Research Article

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## Production of health drink using effective microorganisms and medicinal plant extracts

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### ABSTRACT

*In this present study, medicinal plants namely Centella asiatica and Solanum indicum were collected from the Sirangudi, Madukkur, Thanjavur (Dt), Tamil Nadu. Grapes were collected from the local market at Mannargudi, Thiruvarur (Dt), Tamil Nadu. Intestinal pathogens namely E.coli, and B.subtilis were collected from Microbial Type Culture Collection centre (MTCC) Chandigarh, New Delhi, India. The EM, medicinal plants and grape juice were mixed for the preparation of health drink which were used for the phytochemical analysis, antioxidant activity, alcohol content and antibacterial activity. The phytochemical analysis of health drink is higher than medicinal plant extracts. The antioxidant activity of health drink is higher than medicinal plant extracts. The alcohol content of health drink is lower than yeast. The antibacterial activity of health drink is higher than medicinal plant extracts. The health drink that contains more antioxidant property and low level of alcohol content.*

**Key words:** Effective Microorganisms, Medicinal plant extracts, antioxidant activity, alcohol content.

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### INTRODUCTION

In traditional systems of medicine, the Indian medicinal plants have been used in successful management of various disease in bronchial asthma, chronic fever, cold, cough, malaria, dysentery, convulsions, diabetes, diarrhea, arthritis, skin disease. Plants have been an importance source of medicine for thousands of years. Medicinal plants in Tamil Nadu are cultivated in isolated patches each begin grown in its favourable soil and agroclimatic conditions(1). Medicinal plants are at great interest in the field of biotechnology as most as the pharmaceutical industries depend largely on the utilization at living plants cells for the production of pharmaceutical compounds particularly for alkaloids, flavonoids, tannins, saponins and terpenoids. Drugs may be obtained from various parts of plant. so, an extensive study is required to detect the medical properties of the plant. Several medicinal plants have been tried against pathogenic microorganisms (2). Most EM culture contain from three to fifteen different species of lactic acid bacteria. Phototropic organisms are microbes that are photosynthetic, which can use sunlight to produce energy and energy compounds. All EM culture contain at least 2 or 3 species of phototropic organisms, usually from the extremely powerful and versatile and near magical purple non-sulfur bacteria (PNSB) are family of phototropic microbes, which seem to possess powerful and interesting energy and oxidative effects (3). EM has found application in the many areas especially agriculture, production of health drink, waste water treatment, preparation waste biomass material for bio conversion into fuels such as bio-diesel and other etc. These effective microorganism is used to produce fermented extract from unpolished rice, papaya and see weeds. The effective microorganism fermented extract was claimed to possess has strong anti-oxidation property (4).

The yeast *Saccharomyces cerevisiae* is widely used as a host for the expression of cloned eukaryotic genes. The other yeast in use include *Kluveromyces lactis*, *Schizosaccharomyces cerevisia epombe* and *Pichaperstotis* the yeast in expressing cloned genes. It has a naturally occurring plasmid and strong promoters for efficient expression. The secreted recombinant proteins can be easily isolated. *Saccharomyces cerevisiae* has been in use for several decades in baking and brewing industries. *Saccharomyces cerevisiae* is working yeast. Various strains are used in industry to make bread, beer, wine and industrial alcohol. In some parts of the world sour breads are prepared by using the yeast *Candida lactobacillus*. *Saccharomyces cerevisiae* are collectively referred to as Backers yeast. Backers yeast is commercially available either as a dried powder.

Hence the present study was performed with collection of medicinal plants such as *Centella asiatica* and *Solanum indicum* from Sirangudi, grapes and yeast from local market at Mannargudi, and Effective Microorganism (EM) solution, from Maple Orgtech Ltd, Kolkatta, India. Preparation of health drink by mixing the plant extracts, fermented grape juice and EM solution. To Study the comparative effect of phytochemical properties, antioxidant activity and antibacterial activity of health drink with medicinal plants and alcohol content with yeast.

## EXPERIMENTAL SECTION

### SAMPLE COLLECTION

Medicinal plants such as *Centella asiatica* and *Solanum indicum* were collected from Sirangudi, Madukkur, Thanjavur (Dt), Tamil Nadu. Grapes and yeast (dried) were collected from local market at Mannargudi, Thiruvarur(Dt), Tamil Nadu. Intestinal pathogens namely *Escherichia coli* and *Bacillus subtilis* were collected from Microbial Type Culture Collection centre (MTCC), Chandigarh, New Delhi.

### PREPARATION OF EXTRACT

The mature and undamaged grapes were selected and crushed with mortar to obtain the extract, then it was diluted with chlorine free water and adjusting the initial specific gravity should be between 1.045 and 1.055 while fermenting. The leaves of Vallarai (*Centella asiatica*) and Thudhuvallai (*Solanum indicum*) were ground using mortar with chlorine free water and is filtered using Whatmann filter paper before adding the grape extract.

### EFFECTIVE MICROORGANISM

#### Preparation of Effective Microorganisms

250gm of banana, papaya and pumpkin were collected and chopped into small pieces and transferred into air tight container and mixed with one liter of bore well water, subsequently 250gm of jaggery and one egg was added. The barrel was closed tightly and incubated for 45 days. After 45 days, the white layer was formed on the surface. The fermented EM solution was collected by filtration. It is termed as EM stock solution.

#### Extension (EMe) and activation (EMa) of EM stock solution (5)

For most application, EM stock solution is to be "extended or activated" prior to use.

Extension and activation implies the following materials and steps

One liter of EM stock solution and 1kg of jaggery were mixed with 20 liters of water. The water has been clean and free from chlorine. The container should be of good-grade plastics. For the period of activation, the container was placed in shade at ambient temperature (20-40<sup>0</sup>c) without exposure to strong temperature fluctuations. Extended EM (abbreviated as EMe or EMa) will be ready after 5-10 days. It can be verified by a pH of 3.5 lower and a pleasant sweet sour smell.

### HEALTH DRINK PREPARATION (FERMENTATION)

The grape extracts and plant extracts were mixed well in food grade plastic containers and then it was diluted with chlorine free water and the initial specific gravity should be between 1.045 and 1.05 using a hydrometer. Then the microbial inoculants were added aseptically. The mixture was kept in warm place where the temperature does not exceed 40<sup>0</sup>C; the carbon di-oxide gas formed was released everyday. The mixture was fermented for 7 days until the specific gravity reaches between 1.004. Then, the fermented product was filtered and centrifuged at 10,000 rpm for 20 minutes and the supernatant was used to estimate the alcohol content and total anti-oxidation level.

**PHYTOCHEMICAL ANALYSIS**

Qualitative determination test for tannins, Saponins, Steroids and Quantitative determination test for phenols, alkaloids, tannins, flavonoids were performed (6and7).

**ANTI-OXIDANT ACTIVITY ASSAY BY-PHOSPHOMOLYBDENUM METHOD (8)**

Ascorbic acid standard was pipetted out containing concentration varying from 4-20 µg into series of test tubes and made up to 2 ml with H<sub>2</sub>O. The test tubes were added with 2ml of phosphomolybdenum complex reagent containing 6M H<sub>2</sub>SO<sub>4</sub>, 28mM sodium phosphate, 4mM ammonium molybdate were added and incubated in boiling water bath at 95°C for 90 min. After the samples had cooled at room temperature, the absorbance of the aqueous solution of each was measured at 695nm against blank

**ESTIMATION OF ETHANOL BY POTASSIUM DICHROMATE METHOD (9)**

1ml of various concentration of alcohol (standard) were taken in series of test tubes and added with 25 ml of distilled water and was distilled with liebhig condensor . 15ml of distilled were collected in a 50ml of volumetric flask containing 25ml of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> reagent. These mixtures were kept at 60°C for 30 minutes and measured at 600 nm. A typical blank solution contained 1ml of distilled water and appropriate volume of the same reagent used for standard and it was incubated under the same conditions as the rest of the sample.

**ANTIBACTERIAL ACTIVITY (10)**

The Muller- Hinton agar medium was prepared and autoclaved at 121°C for 15minutes. Then the medium was poured into different petri plates and allowed to solidify. After solidification, the isolated different bacterial species were spread on the petri plates separately. The well was made in the agar plates by using cork borer. In each plate, (40 mg/ 0.ml) of plants extracts were added. The plates without plant extracts were maintained as control. The plate was then incubated at the optimum temperature of test organisms for 16-24 hours. The plates were examined for the zone of inhibition was expressed in millimeter including the diameter of the well.

**STATISTICAL ANALYSIS**

Statistically analysis was performed by calculating Mean ± S.D Gupta (1971).

**RESULTS AND DISCUSSION****SAMPLE COLLECTION**

The medicinal plants, grapes, yeast, and EM solution were collected and used for the analysis of phyto chemical compounds ,antioxidant activity, alcohol content and antibacterial activity.

**Production of health drink (Fermentation)**

Grape extracts (250 ml), plant extract (2%) and EM solution (2%) were mixed together and allowed for fermentation. After fermentation period, the health drink was used for antioxidant, activity alcohol content and antibacterial activity. (11)

**Phytochemical analysis of health drink****Qualitative and Quantitative determination of phyto chemical analysis**

Phyto chemical active compounds such as Tannin, Saponin, and Steroids of health drink and medicinal plants were qualitatively analyzed (Table-I).The health drink contain Alkaloids (4.63±1.32), Phenols (6,72±7.56), Tannin (12±2.4), Flavonoids (9.7±11.43) respectively. The *Tridox procumbens* contain Tannin, Saponin, Triterpenoid, Cardialglycoside, and also analyze the phyto chemical compound such as Tannin, Gallic acid in both qualitative and quantative method. In quantitative analysis the Alkaloids, phenols, Tannin, and Flavonoids were analyzed (12). All the medicinal plants have been contained maximum amount of compound (Table-II). The phytochemical compounds of health drink is similar to medicinal plants. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes (13).

**Antioxidant activity**

The antioxidant activity of health drink were analyzed every day and the result were recorded (Table-III.). The medicinal plant such as *Centella asiatica* and *Solanum indicum* antioxidant activity were analyzed every day and the result were recorded (Table-IV) Antioxidant activity of health drink were compared with medicinal plants namely *Centella asiatica* and *Solanum indicum*. The medicinal plant antioxidant activity is lesser than health drink. The total

antioxidant activity level was also high when compared with medicinal plants this may be organisms present in EM namely phototrophic non-sulphur bacteria (PNSB) converts the free molecules such as phenolics, flavonoids compound into low molecule (14).

### Estimation of alcohol

The alcohol content of health drink were analyzed every day and the result were recorded ((Table-V.) The concentration of ethanol was decreased (6.5%) when EM was used as microbial inoculants it is because of the *Actinobacter* that utilize the ethanol as substrate. *Actinobacter* converts ethanol into acetate and this acetate was transformed into actely CoA and it enters into TCA cycle. (15). The grape extract was fermented for seven days with yeast as inoculants, the alcohol concentration was analyzed every day and the result were recorded (Table-V ). The health drink alcohol content lesser than yeast. Moderate intake of alcohol is associated with a lower risk of developing cardiovascular disease, but this apparent protective effect of alcohol is not fully understood (16).

### Antibacterial activity

Health drink was subjected for antibacterial activity. Antibacterial activity of health drink was analyzed against intestinal pathogens such as *E.coli* and *B.subtilis*. Aqueous extract of health drink and medicinal plants against *E. coli* and *B. subtilis* showed minimum activity (3.1mm±4.21mm) at 25% concentration and maximum activity (8.1mm±9.2mm) at 100% level. Maximum antibacterial activity was observed in aqueous extract of health drink against all tested bacteria namely *E.coli* and *B.subtilis*. Several previous and resent studies have described many important biological activities, particularly antimicrobial activity of *Lawsonia inermis* leaves (17).

Table –I Quantitative analysis of plant extract and Health Drink

S.No	Phyto chemical compound	Plant extract		
		<i>Centella asiatica</i>	<i>Solanum indicum</i>	Health Drink
1	Alkaloid	4.58±0.50	4.13±0.92	3.63±1.32
2	Phenols	7.12±1.13	3.11±0.53	5.72±7.56
3	Tannin	12.±1.20	11.±1.10	11±2.4
4	Flavonoids	9.12±0.72	9.15±0.55	8.7±11.43

Values are expressed as Mean± Standard deviation

TABLE-II Antioxidant level by health drink and medicinal plants

S.No	Number of Days	Centella Asiatica (nm)	Solanum Indicum (nm)	Health Drink (mm)
1	2	100	115	135
2	4	143	165	1.80
3	6	215	230	2.45
4	8	278	290	3.10
5	10	310	355	365
6	12	380	397	415
7	14	400	410	435

TABLE-III Estimation of alcohol from health drink and yeast

S.No	Number of Days	Medicinal plants (nm)	Health Drink (mm)
1	5	100	123
2	10	133	172
3	15	235	235
4	20	312	310
5	25	340	368
6	30	430	402
7	35	450	467

TABLE-IV Antibacterial activity of aqueous extract of health drink and medicinal plant of *E. coli* & *B. subtilis*

S.No	Aqueous extract	Zone of inhibition			
		<i>E.coli</i>		<i>B.subtilis</i>	
		25 %	100 %	25 %	100 %
1	Health Drink	4.3±5.12	8.7±9.7	3.1±4.21	8.1±9.2
2	<i>Centella asiatica</i>	2.3±3.4	6.5±7.2	3.±3.10	6.5±7.3
3	<i>Solanum indicum</i>	2.10±4.5	6.9±7.5	4.5±5.9	5.10±6.2

Values are expressed as Mean± Standard deviation

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**CONCLUSION**

The present study provides evidence to suggest that EM at 2% along with herbal extracts (5%) could generate a beverage with low alcohol content (6.5% v/v) and high antioxidant activity (368 mg/g). This fermented beverage could be used as herbal health drink that contains more antioxidant property and low level of alcohol. However, further studies are needed to evaluate its effectiveness in due course. As the fermentation days increase, there is a reduction in alcohol with concomitant increase in the levels of antioxidant and increase in the acidity. This requires further investigation to confirm the result.

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